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Antiproliferative Effects of Hibernating American Bullfrog (Rana catesbeiana) Blood Plasma on THP-1 Cells

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ANTIPROLIFERATIVE EFFECT OF HIBERNATING AMERICAN BULLFROG, RANA CATESBEIANA, BLOOD PLASMA ON THP-1 CELLS

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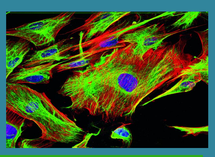


Abstract

Hibernation allows an organism to go dormant during the winter, when food is scarce. Much is unknown physiologically about hibernation, and most current research comes from mammalian models. During hibernation, the cells of mammals are impeded from going through mitosis. 13-lined ground squirrels (Ictidomys tridecemlineatus) and woodchucks (Marmota monax) have a protein, alpha-2-macroglobulin, in their blood plasma, which has significant antiproliferative effects on spleen cells. The goal of this study was to determine if an exothermic organism, the American Bullfrog (Rana catesbeiana), also actively suppresses mitosis while hibernating and if so, determine if it is caused by a component in their blood plasma. Cellular assays using Human THP-1 cells were conducted by incubating cells in different treatments of plasma collected from active and hibernating bullfrogs. After incubation, the cells were either counted to determine if the rate of cell growth had been inhibited, or stained with a dye that attaches to DNA and run through a flow cytometer. Preliminary results of the cell counts were inconclusive regarding whether the rate of growth had been affected by hibernating plasma treatment. Flow cytometry found that the majority of cells in the hibernating plasma treatment were in the M phase of the cell cycle, instead of the G^0 phase. This suggests that the majority of cells had replicated their DNA, but mitosis was halted just afterwards.

Introduction

- Hibernating “warm-blooded” mammals regulate their body temperature and their cells do not undergo division (mitosis)
- This reduces the energetic requirements of the animal during hibernation
- This lack of cell division is regulated by a substance (protein) present in the blood plasma during hibernation
- “Cold-blooded” animals also hibernate, but do not regulate their body temperature

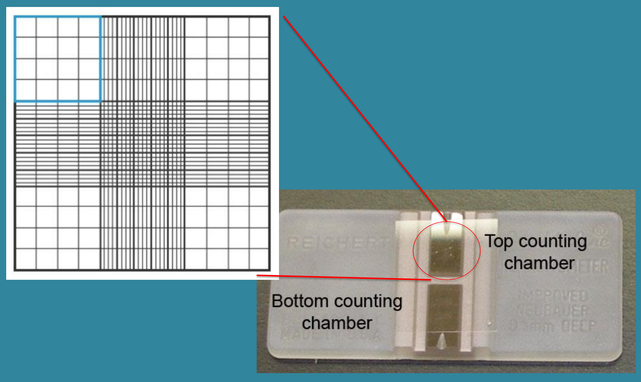
My Question:

- Are “cold-blooded” animals that do not regulate body temperature capable of regulating cell division during hibernation?

Methods & Materials

Frog Plamsa

- 17 bullfrogs, *Rana catesbeiana*, were housed in 20 gallon aquaria, kept in a temperature controlled room with a 12 hour light cycle
- Temperature was decreased gradually until a final temperature of 4°C was reached
- Four bullfrogs were kept at room temperature and served as active bullfrogs
- Blood was drawn from two individuals at a time, at different periods during hibernation and from the four bullfrogs at room temperature



Cell Count Assays

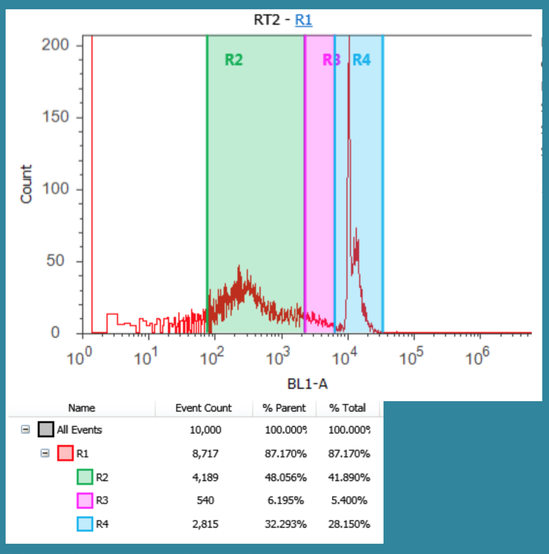
- 1 ml of a human cell line (THP-1) suspension was washed and the cell count was calculated using a hemocytometer
- Cells were placed in either hibernating or active bullfrog plasma for 72 hours in a CO_2 incubator at 37°C
- After incubation, cell numbers were counted using a hemocytometer to determine the effect of differing plasmas on cell proliferation

Flow Cytometer Assays

- 1.5 ml of human cell line suspension was washed
- Cells were placed in either hibernating or active bullfrog plasma for 72 hours in a CO^2 incubator at 37°C
- After incubation, cells were dyed with a stain that attaches to DNA and then run through a flow cytometer to determine the effect of differing plasmas on the cell cycle

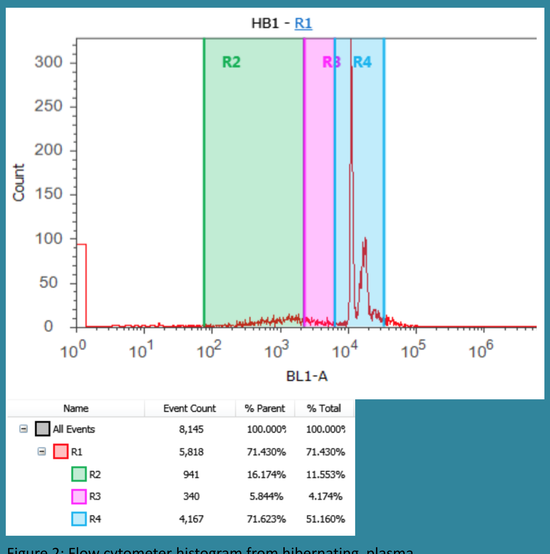
Results

Data collected from the cell count assays were inconclusive due to extreme variance within the data. Data collected from the flow cytometer assays showed that there was a significant difference between the cells grown in active bullfrog plasma and the hibernating bullfrog plasma in the proportionality of cells in the M phase, the stage in which DNA has replicated but the cell has not yet divided. Data also showed that the degree of difference was determined by how far along into hibernation the bullfrog was in when blood was collected.



Name	Event Count	% Parent	% Total
All Events	10,000	100.000%	100.000%
R1	8,717	87.170%	87.170%
R2	4,189	48.056%	41.890%
R3	540	6.195%	5.400%
R4	2,815	32.293%	28.150%

Figure 1: Flow cytometer histogram from room temperature plasma



Name	Event Count	% Parent	% Total
All Events	8,145	100.000%	100.000%
R1	5,818	71.430%	71.430%
R2	941	16.174%	11.553%
R3	340	5.844%	4.174%
R4	4,167	71.623%	51.160%

Figure 2: Flow cytometer histogram from hibernating plasma

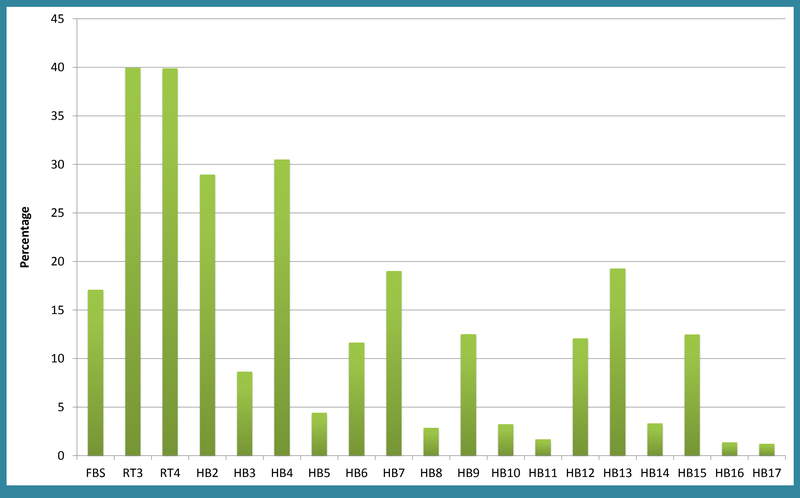


Figure 3: Average percentage of cells in gate 1 of the histogram (green section above) showing cells in G^0 phase

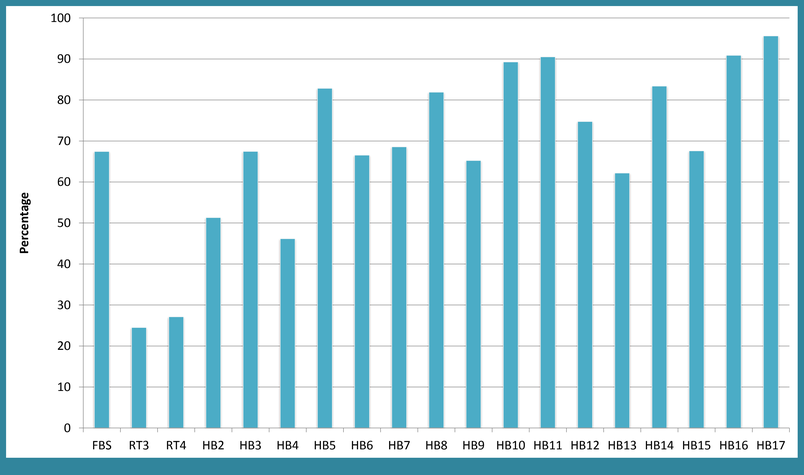


Figure 3: Average percentage of cells in gate 3 of the histogram (blue section above) showing cells in M phase

Conclusion

- Flow cytometry indicates that cold-blooded hibernating animals, like bullfrogs, are capable of regulating the process of cell division
- A greater proportion of cells incubated with hibernating plasma was in the M phase of the cell cycle
- The cells incubated in the active frog plasma had more cells in the G^0 phase, which is the stage of the cell cycle most resting cells are in.
- During hibernation there is a great number of cells that die due to the stress of cold and lack of nutrients. It is speculated that the ability of hibernating frogs to control the process of cell division is a mechanism for replacing cells in the body quickly. By having many cells that have already replicated their DNA the cell can then divide faster upon exiting hibernation.

Reference

Sieckmann DG, Jaffe H, Golech S, Cai D, Hallenbeck JM, McCarron RM. 2014. Anti-lymphoproliferative Activity of Alpha-2-Macroglobulin in the Plasma of Hibernating 13- Ground Squirrels and Woodchucks. Veterinary Immunology and Immunopathology 162: 1-11